

REMARKS/ARGUMENTS

Status of the Claims

Upon entry of the present amendment, Claims 1-7, 13-21, 36-37 and 71-78 are pending. Claims 8-12 have been canceled.

Claims 1-3 have been amended to delete recitation of SEQ ID NO:4. Claims 13-16 have been amended to delete recitation of SEQ ID NO:3. Claims 3 and 16 have been amended to expressly recite 100% identity to the full-length of SEQ ID NOs:8 and 7, respectively. Support is found, for example, on pages 80-82 of the specification.

Support for new Claims 71-74 is found, for example, on page 12, lines 20-23 and on page 14, lines 9-17. Support for new Claims 75-77 is found, for example, on page 21, lines 18-24. Support for new Claim 78 is found, for example, in Figure 3A, and on page 8, lines 8-15.

Objections to the Specification

In accordance with M.P.E.P. § 608.01, Applicants have amended paragraphs 76, 166, 233, and 235 to delete embedded hyperlink or browser-executable code.

Claim Objections

Recitation of Non-Elected Subject Matter

This objection is obviated by canceling the recitation of SEQ ID NO:4 in Claims 1-3 and the recitation of SEQ ID NO:3 in Claims 13-16. Applicants retain the right to rejoin claims reciting the withdrawn SEQ ID NOs should a generic claim be found allowable.

Obviousness-Type Double Patenting

This objection is obviated by cancellation of Claims 8-12. In contrast to the Examiner's assertions, the claims of US Publication No. 2002/0081687 are not applicable to Claims 36 and 37 of the instant application. Claims 36 and 37 depend from Claim 1, which recites an isolated nucleic acid encoding an ABCG8 polypeptide comprising an amino acid sequence that is at least 75% identical to the full-length of SEQ ID NO:8 (*not* SEQ ID NO:6). As evidenced by the

attached BLAST2 sequence alignments,¹ SEQ ID NO:8 and SEQ ID NO:6 share less than 75% amino acid sequence identity, as is recited in amended Claim 1. According to the BLAST2 alignment, SEQ ID NO:8 and SEQ ID NO:6 (called SEQ ID NO:3 in the 2002/0081687 publication) share 28% identity and 48% similarity (“positives”) (*see also*, page 7, lines 13-15 of the specification). Therefore, the claims in the 2002/0081687 publication do not recite a nucleic acid encoding an amino acid sequence that is at least 75% identical to the full-length of SEQ ID NO:8, or obvious therefrom.

Claim Rejections

35 U.S.C. § 112, first paragraph, enablement requirement

The Examiner has rejected Claims 1-21 and 36-37 as allegedly failing the meet the enablement requirement under 35 U.S.C. § 112, first paragraph. Applicants respectfully traverse this rejection because the specification does reasonably provide enablement for isolated nucleic acids which encode an ABCG8 polypeptide, wherein the nucleic acid is at least 80% identical to the full-length of SEQ ID NO:7 or wherein the encoded ABCG8 polypeptide is at least 75% identical to the full-length of SEQ ID NO:8. Applicants further submit that the specification provides guidance to enable those skilled in the art to practice methods of making an ABCG8 polypeptide.

Whereas Claims 1-7 and 13-19 are directed to isolated nucleic acid sequences, the Examiner’s assertions concern isolation of ABCG protein isoforms (paragraph 16 of paper 11). Whereas the claims recite sequence identity, the Examiner’s assertions concern sequence similarity (paragraph 13 of paper 11). Applicants have demonstrated how to make an isolated nucleic acid encoding an ABCG8 polypeptide that is at least 75% identical to the full-length SEQ ID NO:8, as recited in independent Claim 1, because SEQ ID NO:8 (human ABCG8) and SEQ ID NO:4 (mouse ABCG8) are interspecies homologs that encode polypeptides sharing 76% identity.² Likewise, Applicants have also demonstrated how to make an isolated nucleic acid

¹ Exhibit A. Default settings of algorithms (blastn and blastp) provided through the National Center for Biotechnological Information (www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html) were used.

² Exhibit B.

that is at least 80% identical to the full-length SEQ ID NO:7, as recited in Claim 15, because SEQ ID NO:7 (human ABCG8) and SEQ ID NO:3 (mouse ABCG8) share at least 80% identity.³

The Example section on page 65, line 18 through page 72, line 10 demonstrates how Applicants, using well-known methodologies such as sequence databases and standard cloning techniques, identified human ABCG5. Applicants then used the weak nucleic acid sequence identity between human ABCG5 (SEQ ID NO:5) and the *drosophila white* gene to identify human ABCG8 (SEQ ID NO:7) (*see*, page 68, line 14 through page 69, line 12).⁴ In addition to using computer databases, the specification teaches that nucleic acids encoding ABCG8 homologs can be identified from, for example, genomic DNA libraries or expression libraries using techniques well-known to those in the art, including hybridization and amplification (*see*, page 28, line 25 through page 30, line 29). Applicants have shown those of skill in the art how to make, without undue experimentation, an isolated nucleic acid that shares at least 80% sequence identity to SEQ ID NO:7 and which encodes an ABCG8 that shares at least 75% identity to the full-length of SEQ ID NO:8, for instance, by isolating an interspecies homolog.

The specification further teaches how to use the claimed isolated nucleic acids. As the specification teaches, the identified ABCG8 variants have numerous uses in disease treatment and as research tools (*see*, for example page 45, line 3 through page 53, line 19 and page 59, line 1 through page 61, line 31; *see also*, *Integra LifeSciences v. Merck*, 331 F.3d 860, 66 U.S.P.Q.2d 1865). Because the claimed nucleic acids have the testable function of encoding polypeptides that modulate transport of sterols such as cholesterol, they directly can be used to modulate the transcription and translation of ABCG8 proteins and therefore modulate sterol (i.e., cholesterol) transport in a cell (*see*, page 10, lines 24-30, page 32, lines 23-31, page 59, lines 3-13). The claimed nucleic acids also presently can be used to identify modulators of ABCG8 proteins, using methods well known to those in the art. The specification provides detailed guidance for assays for modulators of ABCG8 proteins (page 45, lines 4-26), assays for ABCG8-

³ Exhibit C.

⁴ As shown in Exhibit A, BLAST2 analysis between SEQ ID NO:5 and SEQ ID NO:7 found no significant sequence similarity.

interacting compounds (page 45, line 27 through page 51, line 27), and assays for ABCG8 protein activity (page 51, line 27 through page 53, line 19). Modulation of ABCG8 transcription and/or expression can be significant for the treatment of numerous diseases, including sitosterolemia, atherosclerosis, hyperlipidemia, gall stones (*e.g.*, cholesterol stones) hypercholesterolemia (*e.g.*, familial hypercholesterolemia), coronary heart disease, HDL deficiency, nutritional deficiency, arthritis, xanthomas, and hemolytic anemia. (*see*, page 60, lines 3-10).

With regard to Claim 36, which recites a method of making an ABCG8 polypeptide, isolation of the polypeptide is not an element of the claim. Further, because the claim recites making an ABCG8 polypeptide that is at least 75% identical to the full-length of SEQ ID NO:8, the expressed protein by definition has identifying topological features that facilitate predictability of expression, including an ATP domain, a hydrophobic domain comprising six transmembrane segments, a Walker A motif, a Walker B motif a Signature C motif and other signature sequences typical of ABC transporter (page 11, lines 26-30; page 15, lines 18-21). Further, assaying for protein activity of an ABCG8 variant does not require undue experimentation, but can be accomplished using a routine *in vitro* cholesterol (or other lipid) transport assay well known to those of skill in the art (for instance, using radioactively labeled cholesterol; page 52, lines 8-13). Animal-based models for assaying ABCG8 activity are also routine (page 52, lines 14-20).

With regard to Claim 37, which recites the further step of recovering the polypeptide from the host cell or cellular extract, the specification provides detailed guidance for protein purification on page 35, line 4 through page 38, line 2. There is no reason for one of skill in the art to believe that recovering one species of an ABCG8 polypeptide within the genus of ABCG8 polypeptides that are at least 75% identical to the full-length of SEQ ID NO:8 will be unpredictably different from another species, because all ABCG8 species claimed by definition possess the same distinguishing topological features. The recovery steps, as outlined on page 35, line 4 through page 38, line 2, are not highly unpredictable, but routine and methodical to those of skill in the art. Furthermore, ABC transporters constitute a group of evolutionary highly conserved cellular transmembrane transport proteins (*see*, Schmitz *et al.*, *Curr Opin Lipidol*

11:493 (2000)), and others have recovered ABC transporter proteins from cells with introduced nucleic acids that express ABC transporter proteins (*see*, Schneider *et al.*, *Protein Expr Purif* 6:10 (1995) and Meyer *et al.*, *FEBS Lett* 351:443 (1994)).⁵

The Examiner's assertion that point mutations can eliminate or lessen ABCG8 activity is conceivable. However, those of skill in the art could reasonably expect that more likely than not, an ABCG8 polypeptide at least 75% identical to the full-length of SEQ ID NO:8 would function as a sterol transporter because sitosterolemia is a rare disease (page 1, lines 7-10). Moreover, as amended, Claim 1 now recites "wherein said polypeptide acts to effect sterol transport" (*see*, page 8, lines 29-32 and page 11, lines 21-23) and, thus, Claim 1 is only directed to isolated nucleic acids encoding an ABCG8 polypeptide, wherein the ABCG8 polypeptide acts to effect sterol transport. Also, as discussed above, assaying for protein activity of an ABCG8 variant can be accomplished using a routine *in vitro* labeled cholesterol (or other lipid) transport assay that can efficiently be carried out with numerous parallel samples, or even using high-throughput screening (page 10, lines 19-23). Routine screening, even if time consuming, does not constitute undue experimentation (*In re Wands*, 858 F.2d 731).

For the foregoing reasons, Applicants respectfully submit that they have shown those of skill in the art how to make and use isolated nucleic acid encoding an ABCG8 protein having at least 75% amino acid sequence identity to the full-length of SEQ ID NO:8. Accordingly, the Examiner is respectfully requested to withdraw this rejection.

35 U.S.C. § 112, first paragraph, written description requirement

The Examiner has rejected Claims 1, 8, 12 and 15 as allegedly failing the written description requirement. Claims 8 and 12 have been cancelled. Applicants respectfully traverse this rejection because Applicants were in possession of the claimed isolated nucleic acid sequences, and because the ABCG8 polypeptides encoded by the nucleic acid sequences share common structure and function.

The specification shows that Applicants were in possession of the claimed isolated nucleic acid sequences at the time of filing. Amended Claim 1 recites an isolated nucleic acid

⁵ Abstracts attached as Exhibit D.

encoding an ABCG8 polypeptide comprising an amino acid sequence that is at least 75% identical to the full-length of an amino acid sequence as set forth in SEQ ID NO: 8.

SEQ ID NO:8 and SEQ ID NO:4 are interspecies homologs encoding ABCG8 polypeptides and sharing at least 75% amino acid sequence identity.⁶ Amended Claim 15 recites an isolated nucleic acid sequence (encoding an ABCG8 polypeptide) that comprises a nucleotide sequence at least 80% identical to SEQ ID NO:7. SEQ ID NO:7 and SEQ ID NO:3 are interspecies homologs encoding ABCG8 polypeptides and sharing at least 80% nucleic acid identity.⁷

Claims 1 and 15 are not directed to *any* isolated nucleic acid sequence encoding a protein having at least 80% sequence identity to the full-length of SEQ ID NO:8 or SEQ ID NO:7, but to a nucleic acid sequence encoding an *ABCG8* polypeptide. The recitation of an ABCG8 polypeptide defines particular structure, as depicted in Figure 1, and particular topological elements, including a transport unit, an ATP binding domain, a hydrophobic domain comprising six transmembrane segment, a Walker A motif, a Walker B motif and a Signature C motif (*see*, page 11, lines 26-30, page 15, lines 18-20).

As amended, Claims 1 and 15 further correlate structure with function. Amended Claim 1 now recites “wherein said polypeptide acts to effect sterol transport” (*see*, page 8, lines 29-32 and page 11, lines 21-23).

Therefore, in accordance with the Examiner’s concerns, Claims 1 and 15 structurally and functionally define the claimed genus. SEQ ID NOs: 3, 4, 7 and 8 demonstrate that Applicants were objectively in possession of at least two representative interspecies homologs within the scope of Claims 1 and 15.

35 U.S.C. § 102(e) over Tang *et al.* (WO 02/40541 A2)

This rejection, as it applies to Claims 1-2, 4-7, 13-15 and 36-37, is obviated by the attached Declaration under 37 C.F.R. § 1.131, which establishes conception and reduction to practice of the claimed invention before October 27, 2000.

⁶ Exhibit B.

⁷ Exhibit C.

Applicants respectfully submit that this rejection does not apply to amended Claims 3 and 16. A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently, in a single reference. M.P.E.P. § 2131. Tang *et al.* does not teach or suggest an isolated nucleic acid encoding an ABCG8 polypeptide having 100% identity to the full length of SEQ ID NO:8, as is recited in Claim 3. Further, Tang *et al.* does not teach or suggest a nucleic acid having 100% identity to the full length of SEQ ID NO: 7, as is recited in Claim 16. Therefore, Tang *et al.* does not properly anticipate Claim 3 or Claim 16.

35 U.S.C. § 102(e) over Patel *et al.* (WO 02/27016 A2)

This rejection is obviated by the cancellation of Claims 8-12. For the reasons discussed above in the context of double-patenting, Patel further does not anticipate Claims 36 and 37, which depend from Claim 1, because SEQ ID NO:8 and SEQ ID NO:6 share less than 80% amino acid sequence identity.

35 U.S.C. § 102(e) over Tian *et al.* (WO 02/79292 A2)

This rejection is obviated by the cancellation of Claims 8-12. For the reasons discussed above in the context of double-patenting, Tian *et al.* does not anticipate Claims 36 and 37, which depend from Claim 1, because SEQ ID NO:8 and SEQ ID NO:6 share less than 80% amino acid sequence identity.

CONCLUSION

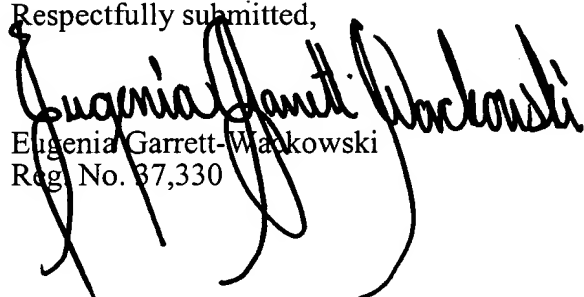
In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

Appl. No. 09/989,981
Amdt. dated February 9, 2004
Reply to Office Action of August 8, 2003

PATENT

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 925-472-5000.

Respectfully submitted,


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Attachments
EGW:jlw:tc

60026078 v2

**Blast 2 Sequences results**

PubMed

Entrez

BLAST

OMIM

Taxonomy

Structure

BLAST 2 SEQUENCES RESULTS VERSION BLASTN 2.2.6 [Apr-09-2003]

Match: 1 Mismatch: -2 gap open: 5 gap extension: 2
x_dropoff: 50 expect: 10.000 wordsize: 11 Filter ☒ Align

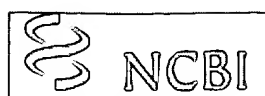
Sequence 1 lc|seq_1 Length 2340 SEQ ID NO: 5

Sequence 2 lc|seq_2 Length 2669 SEQ ID NO: 7

No significant similarity was found

EXHIBIT

A



Blast 2 Sequences results

PubMed

Entrez

BLAST

OMIM

Taxonomy

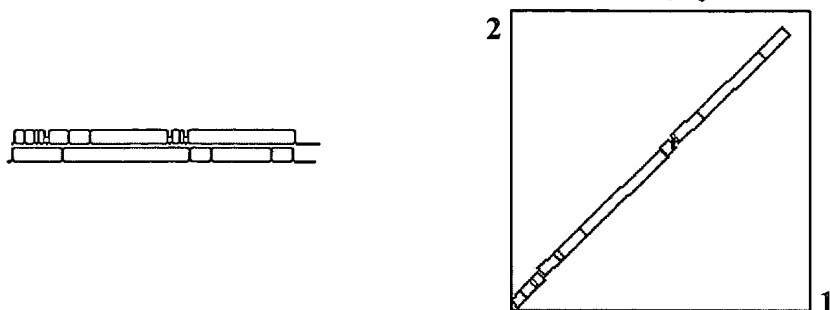
Structure

BLAST 2 SEQUENCES RESULTS VERSION BLASTP 2.2.6 [Apr-09-2003]

Matrix: BLOSUM62 ☐ gap open: 11 gap extension: 1
 x_dropoff: 50 expect: 10.000 wordsize: 3 Filter ☒ Align

Sequence 1 lc|seq_1 Length 651 (1 .. 651) SEQ ID NO: 6

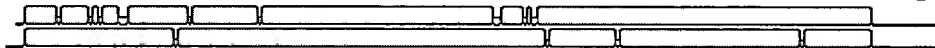
Sequence 2 lc|seq_2 Length 673 (1 .. 673) SEQ ID NO: 8



NOTE: The statistics (bitscore and expect value) is calculated based on the size of nr database

Score = 244 bits (622), Expect = 8e-63

Identities = 174/615 (28%), Positives = 303/615 (48%), Gaps = 30/615 (4%)



Query: 8 TPGGSMGLQVNRGSQSSLEGAPAT-APEPHSLGILHASYSVSHRVR-PWWD-ITSCRQQW 64
 TP + GLQ S S T + +P++L + +Y V + PW++ + + W
 Sbjct: 16 TPQDTSGQLQDRLFSSSESDNSLYFTYSGQPNTLEVRDLNYQVDLASQVPWFELAQFKMPW 75

Query: 65 TRQI-----LKDVSILYVESGQIMCILGSSSGSGKTTLLDAMSGRLGRAGTFL-GEVYV 115
 T ++++S V SGQ++ I+GSSG G+ +LLD ++GR G G G++++
 Sbjct: 76 TSPSCQNSCELGIQNLISFKVRSGQMLAIIGSSGCGRASLLDVITGR-GHGGKIKSGQIWI 134

Query: 116 NGRALRREQFQDCFSYVLQSDTLLSSLTVRETLHYTALLAIRRG-NPGSFQKKVEAVMAE 174
 NG+ + + C ++V Q + LL +LTVRETL + A + + R + K+VE V+AE
 Sbjct: 135 NGQPSSPQLVRKCAHVRQHNQLLPNLTVRETLAFIAQMRLPRTFSQAQRDKRVEDVIAE 194

Query: 175 LSLSHVADRLIGNYSLGISTGERRRVSIAAQLLQDPKVMLFDEPTTGLDCMTANQIVVL 234
 L L AD +GN + G+S GERRRVS I QLL +P +++ DEPT+GLD TA+ +V
 Sbjct: 195 LRLRQCADTRVGNMYVRGLSGGERRRVSIGVQLLWNPGLILDEPTSGLDSTAHNLVKT 254

Query: 235 LVELARRNRIVLTIHQPRSELFQFDKIAILSFGELIFCGTPAEMLDFFNDCGYPCPEH 294
 L LA+ NR+V++++HQPRS++F+LFD + +++ G I+ G M+ +F GYPCP +
 Sbjct: 255 LSRLAKGNRLVLISLHQPRSDIFRLFDLVLLMTSGTPIYLGAAQHMVQYFTAIGYPCPRY 314

Query: 295 SNPFDFYMDLTSVDTQSKEREIETSKRVQMIESAYKKS-----ICHKTLKNIERMKHL 348
 SNP DFY+DLTS+D +S+E+E+ T ++ Q + + + + + K+++ +
 Sbjct: 315 SNPADFVYDLTSIDRRSREQELATREKAQSLAALFLEKVRDLDDFLWKAETKDLDEDTCV 374

Query: 349 KT--LPM----VPFKTKDSPGVFSKXXXXXXXXXXXXXXXXKLAVITRLLQNLIMGXXXXX 402
 ++ P+ +P TK PG + + ++ + +M

Sbjct: 375 ESSVTPLDTNCLPSP TK-MPGAVQQFTTLIRRQISNDFRDLPTLLIHGAEACLSMTIGF 433

Query: 403 XXXXXXSNVLKGAIQDRVGLLYQFVGATPYTGMLNAVNLFPVLRAVSDQESQDGLYQKWQ 462
 S ++ + D LL+ P+ +L+ ++ RA+ E +DGLY

Sbjct: 434 LYFGHGS--IQLSFMDTAALLFMIGALIPFNVILDVISKYSERAMLYYELEDGLYTTGP 491

Query: 463 MMLAYALHVLFPFSVVATMIFSSVCYWTGLHPEVARFGYFSAALLAPHLIGEFLLVLLG 522
 A L LP +I+ YW L P + F + + L

Sbjct: 492 YFFAKILGELPEHCAYIIIIYGMPYWLANLRPGLQPFLHLLVWLTVFCCRIMALAAAA 551

Query: 523 IVQNPNIIVNSVVALLSIAGVLVSGFLRNIQEMPIPFKIISYFTFQKYCSEILVVNEFYG 582
 ++ ++ + L + L G GF+ N+ + IS +F ++C E L+ +F

Sbjct: 552 LLPTFHMASFFSNALYNSFYLAG-GFMINLSSLWTVPAWISKVSFLRWCFEGLMKIQFSR 610

Query: 583 LNFTCGSSNVSVTTN 597

+ N+++ +

Sbjct: 611 RTYKMPLGNLTI AVS 625

CPU time: 0.04 user secs. 0.01 sys. secs 0.05 total secs.

Lambda	K	H
0.324	0.138	0.408

Gapped

Lambda	K	H
0.267	0.0410	0.140

Matrix: BLOSUM62

Gap Penalties: Existence: 11, Extension: 1

Number of Hits to DB: 3057

Number of Sequences: 0

Number of extensions: 251

Number of successful extensions: 4

Number of sequences better than 10.0: 1

Number of HSP's better than 10.0 without gapping: 1

Number of HSP's successfully gapped in prelim test: 0

Number of HSP's that attempted gapping in prelim test: 0

Number of HSP's gapped (non-prelim): 1

length of query: 651

length of database: 529,226,880

effective HSP length: 135

effective length of query: 516

effective length of database: 529,226,745

effective search space: 273081000420

effective search space used: 273081000420

T: 9

A: 40

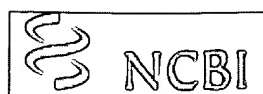
X1: 15 (7.0 bits)

X2: 129 (49.7 bits)

X3: 129 (49.7 bits)

S1: 41 (22.0 bits)

S2: 79 (35.0 bits)



Blast 2 Sequences results

PubMed

Entrez

BLAST

OMIM

Taxonomy

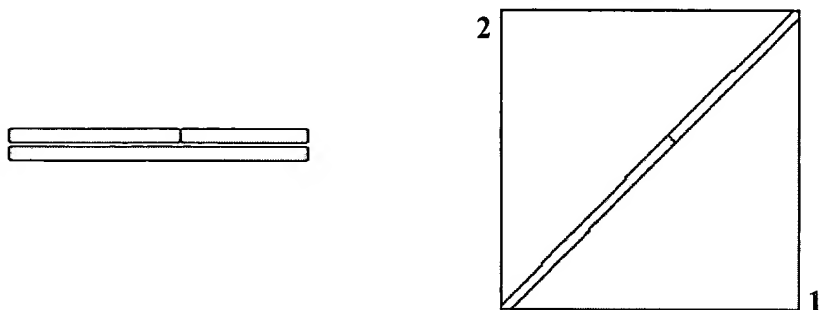
Structure

BLAST 2 SEQUENCES RESULTS VERSION BLASTP 2.2.6 [Apr-09-2003]

Matrix: BLOSUM62 ☐ gap open: 11 gap extension: 1
 x_dropoff: 50 expect: 10.000 wordsize: 3 Filter ☒ Align

Sequence 1 lcl|seq_1 Length 672 (1 .. 672) SEQ ID NO: 4 (mouse ABCG8)

Sequence 2 lcl|seq_2 Length 673 (1 .. 673) SEQ ID NO: 8 (human ABCG8)



NOTE: The statistics (bitscore and expect value) is calculated based on the size of nr database

Score = 1021 bits (2641), Expect = 0.0

Identities = 512/673 (76%), Positives = 560/673 (83%), Gaps = 1/673 (0%)

```

Query: 1  MAEKTKEETQLWNGTVLQDASGLQDSLFSSES DNSLYFTYSGQSN TLEV RDLT YQVDIAS 60
          MA K  EE  L  G   QD SGLQD LFSSES DNSLYFTYSGQ NTLEV RD L YQVD+AS
Sbjct: 1  MAGKAAEERGLPKGATPQDTSGLQDRLFSSES DNSLYFTYSGQPN TLEV RD LNYQVDLAS 60

Query: 61 QVPWF EQLAQFKIPWRSHSSQDSC ELGIRNLSFKVRS GQMLAIIGSSGCGRASLLDVITG 120
          QVPWF EQLAQFK+PW S S Q+SCE LGI+NLSFKVRS GQMLAIIGSSGCGRASLLDVITG
Sbjct: 61 QVPWF EQLAQFKMPWTSPSCQNSCE LGIQNLSFKVRS GQMLAIIGSSGCGRASLLDVITG 120

Query: 121 RGHGGKMKSGQIWINGQPSTPQLVRKCVAHVRQH DQLLPNLTVRETLAFIAQMRLPRTFS 180
          RGHGGK+KSGQIWINGQPS+PQLVRKCVAHVRQH+QLLPNLTVRETLAFIAQMRLPRTFS
Sbjct: 121 RGHGGKIKSGQIWINGQPSSPQLVRKCVAHVRQHNQLLPNLTVRETLAFIAQMRLPRTFS 180

Query: 181 QAQRDKRVEDVIAELRLRQC ANTRVGN TYXXXXXXXXXXXXXXXXX QLLWNP GILILDEPT 240
          QAQRDKRVEDVIAELRLRQCA+TRVGN Y QLLWNP GILILDEPT
Sbjct: 181 QAQRDKRVEDVIAELRLRQC ADTRVGN MYVRGLSGGERRRVSIGVQLLWNP GILILDEPT 240

Query: 241 SGLDSFTA HNLVT TSLRLAKGNRLVLISLHQPRSDIFRLFDLVLLMTSGTPIYLGAAQQM 300
          SGLDSFTA HNLV TSLRLAKGNRLVLISLHQPRSDIFRLFDLVLLMTSGTPIYLGAAQ M
Sbjct: 241 SGLDSFTA HNLVKTSLRLAKGNRLVLISLHQPRSDIFRLFDLVLLMTSGTPIYLGAAQHM 300

Query: 301 VQYFTSIGHPCPRYSNPADFYVDLTSIDRRSKEREVATVEKAQSLAALFLEKVQGFD DFL 360
          VQYFT+IG+PCPRYSNPADFYVDLTSIDRRS+E+E+AT EKAQSLAALFLEKV+ DDFL
Sbjct: 301 VQYFTAIGYPCPRYSNPADFYVDLTSIDRRSREQELATREKAQSLAALFLEKVRDLDDFL 360

Query: 361 WKAEAKELNTSTHTVSLT LTQD TDC-GTAVELPGMIEQFSTLIRRQISNDFRDLPTLLIH 419
          WKAE K+L+ T S DT+C + ++PG ++QF+TLIRRQISNDFRDLPTLLIH
  
```

EXHIBIT

B

Sbjct: 361 WKAETKDLDEDTCESSVTPLDTNCLPSPTKMPGAVQQFTTLIRRQISNDFRDLPTLLIH 420

Query: 420 GSEACLSLIIGFLYVGHGALQLSFMDTAALLFMIGALIPFNVILDVVSCHSERSMLYY 479
 G+EACLS+ IGFLY+GHG++QLSFMDTAALLFMIGALIPFNVILDV+SKC+SER+MLYY

Sbjct: 421 GAEACLSMTIGFLYFGHGSIQLSFMDTAALLFMIGALIPFNVILDVISKCYSERAMLYY 480

Query: 480 ELEDGLYTAGPYFFAKILGELPEHCAYVIIYAMPIYWLTLNLRPVPEXXXXXXXXXXXXXXX 539
 ELEDGLYT GPYFFAKILGELPEHCAY+IIY MP YWL NLRP +

Sbjct: 481 ELEDGLYTTGPYFFAKILGELPEHCAYIIYGMPTYWLANLRPGLQPFLHFLLVWL VVF 540

Query: 540 CCRTMALAASAMLPFTHMSSFFCNALYNSFYLTAGFMINLDNLWIVPAWISKLSFLRWCF 599
 CCR MALAA+A+LPFTHM+SFF NALYNSFYL GFMINL +LW VPAWISK+SFLRWCF

Sbjct: 541 CCRIMALAAAALLPFTFHMASFFSNALYNSFYLAGGFMINLSSLWTPAWISKVSFLRWCF 600

Query: 600 SGLMQIQFNHLYTTQIGNFTFSILGDTMISAMDLNSHPLYAIYLIVIXXXXXXXXXXXXXXXX 659
 GLM+IQF+ Y +GN T ++ GD ++SAM+L+S+PLYAIYLIVI

Sbjct: 601 EGLMKIQFSRRTYKMPGLNLTIAVSGDKILSAMELDSYPLYAIYLIVIGLSGGFMVLYYV 660

Query: 660 XXXXXXQKSIQDW 672
 QK QDW

Sbjct: 661 SLRFIKQKPSQDW 673

CPU time: 0.04 user secs. 0.00 sys. secs 0.04 total secs.

Lambda	K	H
0.324	0.137	0.416

Gapped

Lambda	K	H
0.267	0.0410	0.140

Matrix: BLOSUM62

Gap Penalties: Existence: 11, Extension: 1

Number of Hits to DB: 3584

Number of Sequences: 0

Number of extensions: 237

Number of successful extensions: 3

Number of sequences better than 10.0: 1

Number of HSP's better than 10.0 without gapping: 1

Number of HSP's successfully gapped in prelim test: 0

Number of HSP's that attempted gapping in prelim test: 0

Number of HSP's gapped (non-prelim): 1

length of query: 672

length of database: 529,226,880

effective HSP length: 135

effective length of query: 537

effective length of database: 529,226,745

effective search space: 284194762065

effective search space used: 284194762065

T: 9

A: 40

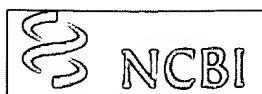
X1: 15 (7.0 bits)

X2: 129 (49.7 bits)

X3: 129 (49.7 bits)

S1: 40 (21.5 bits)

S2: 79 (35.0 bits)



Blast 2 Sequences results

PubMed

Entrez

BLAST

OMIM

Taxonomy

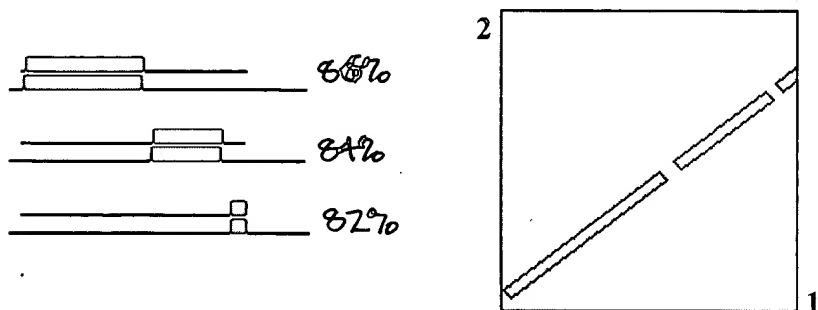
Structure

BLAST 2 SEQUENCES RESULTS VERSION BLASTN 2.2.6 [Apr-09-2003]

Match: 1 Mismatch: -2 gap open: 5 gap extension: 2
x dropoff: 50 expect: 10.000 wordsize: 11 Filter ☒ Align

Sequence 1 | cl|seq_1 Length 2019 (1 .. 2019) SEQ ID NO: 3 (mouse ABCG8)

Sequence 2 lc|seq_2 Length 2669 (1 .. 2669) SEQ ID NO:7 (human ABCG8)



NOTE: The statistics (bitscore and expect value) is calculated based on the size of nr database

NOTE: If protein translation is reversed, please repeat the search with reverse strand of the query sequence

Score = 1210 bits (629), Expect = 0.0

Identities = 909/1049 (86%)

Strand = Plus / Plus

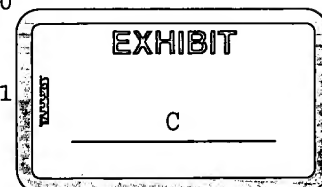
[illegible]

```
Query: 112   ttcacctacagtggtcagttccaacactctggaggtcagagatctcacctaccaggtggac 171
            |||||
Sbjct: 211   ttcacctacagtggccagcccaacaccctggaggtcagagacctcaactaccaggtggac 270
```

Query: 172 atcgctctcaggtgccttggtttgagcagctggctcagtccaagataccctggaggctc 231
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct: 271 ctggctctcaggtcccttggtttgagcagctggctcagtccaagatgccctggacatct 330

Query: 232 catagcagccaagactcctgtgagctgggcatccgaaatctaagcttcaaagtgaggagt 291
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct: 331 cccagctgccagaattcttgtgagctgggcatccagaaccttaagcttcaaagtgagaagt 390

Query: 292 ggacagatgctggccatcatagggagctcaggctgctgggagagcctcactactcgacgtg 351



1/22/2004

Sbjct: 1111 accagggagaaggctcagtcactcgcagccctgtttctagaaaaagtgcgtgacttagat 1170

Query: 1072 gactttctgtggaaagctgaggcaaagga 1100

||||||| ||||||| ||| | |||||

Sbjct: 1171 gactttctatggaaagcagagacgaagga 1199

Score = 654 bits (340), Expect = 0.0

Identities = 534/631 (84%)

Strand = Plus / Plus

Query: 1187 agcagttttccaccctgatccgtcgtcagatttccaatgacttccgggacctgcccacgc 1246

||||||| | || ||||||||||||||||| ||||||||| ||||||||| ||

Sbjct: 1289 agcagtttacgacgctgatccgtcgtcagatttccaacgacttccgagacctgcccaccc 1348

Query: 1247 tgctcattcatgggtcggaaagcctgcctgatgtccctcatcattggcttcctttactacg 1306

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Sbjct: 1349 tcctcatccatggggcggaggcctgtctgatgtcaatgaccatcggcttcctctattttg 1408

Query: 1307 gccatggggccaagcagctctccttcatggacacagcagccctccttctcatgatagggg 1366

||||||| || ||||||||||||||||| ||||| ||||||| | ||||||||| |||

Sbjct: 1409 gccatgggagcatccagctctccttcatggatacagccgacctcttggtcatgatcggtg 1468

Query: 1367 cgctcattcctttcaatgtcatcctggatgtcgtctccaaatgtcactcggagaggtcaa 1426

| ||||| ||||||| ||||| ||||||||| ||||||||| ||||| ||||||| |||

Sbjct: 1469 ctctcatccctttcaacgtcattctggatgtcatctccaaatgttactcagagagggcaa 1528

Query: 1427 tgctgtactatgagctggaagacgggctgtacactgctggtccttatttctttgccaaga 1486

|||| ||||||| ||||||||| ||||||| ||||||| ||||||| ||||||| ||

Sbjct: 1529 tgctttactatgaactggaagacgggctgtacaccactgggtccatatttctttgccaaga 1588

Query: 1487 tcctaggagaattgccggagcactgtgcctacgtcatcatctacgcgatgcccatctact 1546

|||| | || | ||||||||| ||||||||| ||||||||| ||||||||| |||||

Sbjct: 1589 tcctcggggagcttcgggagcactgtgcctacatcatcatctacgggatgccacactact 1648

Query: 1547 ggctgacaaacctgcggcccgctgcctgagctcttccttctacacttctgctcgtgtggt 1606

||||| | ||||||| |||| | || ||||||| || ||||||||| |||||||

Sbjct: 1649 ggctggccaacctgaggccaggcctccagcccttctgctgcacttctgctggtgtggc 1708

Query: 1607 tgggtggtcttctgctgcaggacatggccctggctgcctctgccatgctgccaccttcc 1666

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Sbjct: 1709 tgggtggtcttctggtgcaggattatggccctggccgcccggccctgctccccaccttcc 1768

Query: 1667 acatgtcctccttcttctgcaatgccctctacaactccttctaccttactgccggcttca 1726

||||| ||||||| ||||||| ||||||||| ||||||||| ||||| || |||||||

Sbjct: 1769 acatggcctccttcttcagcaatgccctctacaactccttctacctcgccgggggttca 1828

Query: 1727 tgataaacttgacaacctgtggatagtgacctgatggatctccaagctgtcgttctcc 1786

|||||
Sbjct: 1829 tgataaacttgagcagcctgtggacagtgcccgcgtggatttccaaagtgtccttcctgc 1888

Query: 1787 ggtggtgcttctcggggctgatgcagattca 1817

|||||
Sbjct: 1889 ggtggtgttttgaagggctgatgaagattca 1919

Score = 119 bits (62), Expect = 2e-23
Identities = 110/134 (82%)
Strand = Plus / Plus

Query: 1886 tcagtgccatggacctgaactcgcacccactctatgcgatctacctcattgtcatcggca 1945

|||||
Sbjct: 1988 tcagtgccatggagctggactcgtaccctctctacgccatctacctcatcgtcattggcc 2047

Query: 1946 tcagctacggcttctcgttctgtactatctatccttgaagctcatcaaacagaagtcaa 2005

|||||
Sbjct: 2048 tcagcgggtggcttcatggctcgtactacgtgtccttaaggttcatcaaacagaaaccaa 2107

Query: 2006 ttcaagactggtga 2019

|||||
Sbjct: 2108 gtcaagactggtga 2121

CPU time: 0.03 user secs. 0.01 sys. secs 0.04 total secs.

Lambda	K	H
1.33	0.621	1.12

Gapped

Lambda	K	H
1.33	0.621	1.12

Matrix: blastn matrix:1 -2

Gap Penalties: Existence: 5, Extension: 2

Number of Hits to DB: 7

Number of Sequences: 0

Number of extensions: 7

Number of successful extensions: 4

Number of sequences better than 10.0: 1

Number of HSP's better than 10.0 without gapping: 1

Number of HSP's successfully gapped in prelim test: 0

Number of HSP's that attempted gapping in prelim test: 0

Number of HSP's gapped (non-prelim): 4

length of query: 2019

length of database: 9,775,304,123

effective HSP length: 26

effective length of query: 1993

effective length of database: 9,775,304,097

effective search space: 19482181065321

effective search space used: 19482181065321

T: 0

A: 0

X1: 6 (11.5 bits)

X2: 26 (50.0 bits)
S1: 12 (23.8 bits)
S2: 21 (41.1 bits)



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☐ 1: Curr Opin Lipidol. 2000 Oct; 11(5): 493-501.

Related Articles, Links

**ABC transporters in cellular lipid trafficking.****Schmitz G, Kaminski WE, Orso E.**

Institute for Clinical Chemistry and Laboratory Medicine, University of Regensburg, Germany. gerd.schmitz@klinik.uni-regensburg.de

ATP-binding cassette (ABC) transporters constitute a group of evolutionary highly conserved cellular transmembrane transport proteins. Recent work has implicated ABC transporters in cellular transmembrane lipid transport and hereditary diseases have been causatively linked to defective ABC transporters translocating lipid compounds. The emerging concept that a defined subset of ABC transporters is intimately involved in cellular lipid trafficking has recently been substantiated convincingly by the finding that ABCA1 plays a central role in the regulation of HDL metabolism and macrophage targeting to the RES or the vascular wall. Differentiation dependent expression of a large number of ABC transporters in monocytes/macrophages and their regulation by sterol flux render these transporter molecules potentially critical players in atherogenesis and other chronic inflammatory diseases.

Publication Types:

- Review
- Review, Tutorial

PMID: 11048892 [PubMed - indexed for MEDLINE]

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FULL-TEXT ARTICLE**Functional purification of a bacterial ATP-binding cassette transporter protein (MalK) from the cytoplasmic fraction of an overproducing strain.****Schneider E, Linde M, Tebbe S.**

Arbeitsgruppe Mikrobiologie, Fachbereich Biologie/Chemie, Universitat Osnabruck, Germany.

The malK gene of *Salmonella typhimurium* encoding the ATP-hydrolyzing subunit of the ATP-Binding Cassette (ABC) transporter for maltose was subcloned into the pRSET5d expression vector. Subsequently, the resulting plasmid (pES67) was introduced into *Escherichia coli* strain BL21(DE3)/pLysS. When strain BL21-(DE3)/pLysS/pES67 was grown at 30 degrees C in a tryptone-phosphate medium (J.T. Moore, A. Uppal, F. Maley, and G. F. Maley, *Protein Expression Purif.* 4, 160-163, 1993), the addition of isopropyl beta-thiogalactoside resulted in the synthesis of large amounts of MalK protein. After cell disruption about 60% of MalK was recovered with the soluble (cytoplasmic) fraction. The protein was purified by ion exchange chromatography and dye ligand affinity chromatography. The purified MalK protein displayed enzymatic properties similar to those of a preparation purified and renatured from inclusion bodies (S. Morbach, S. Tebbe, and E. Schneider, *J. Biol. Chem.* 268, 18617-18621, 1993). Thus, our results disprove the view that the biochemical properties of a protein renatured from inclusion bodies might be artefactual. In addition, we provide further evidence that the modification of growth conditions and the use of a T7 expression system can be a useful approach to overcome at least in part the formation of inclusion bodies.

PMID: 7756834 [PubMed - indexed for MEDLINE]

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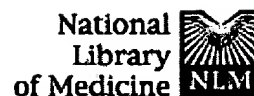
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☐ 1: FEBS Lett. 1994 Sep 12; 351(3): 443-7.[Related Articles, Links](#)

Functional expression and purification of the ABC transporter complex associated with antigen processing (TAP) in insect cells.

Meyer TH, van Endert PM, Uebel S, Ehring B, Tampe R.

Max-Planck-Institut fur Biochemie, Martinsried, Germany.

Using the baculovirus expression system the gene products of human tap1 and tap2 were over-expressed as wild-type as well as oligohistidine fusion proteins in *Spodoptera frugiperda* (Sf9) insect cells. Both gene products were co-expressed within the same cells and were found enriched in microsomal membranes. Immunoprecipitation and immobilized metal affinity chromatography revealed complex formation between TAP1 and TAP2. The expressed TAP complex was shown to be functional by peptide translocation into microsomes of Sf9 cells. Peptide transport strictly requires TAP1 and TAP2 as well as ATP. For the first time the functional expression of the human TAP complex in insect cells has been demonstrated, indicating that additional cofactors of a highly developed immune system are not essential for peptide transport across microsomal membranes.

PMID: 8082812 [PubMed - indexed for MEDLINE]

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